Appendix B

Attached are (i) an English translation of the foreign priority document, Italian Application No. MI2003A001156, filed June 9, 2003, and (ii) a certificate of translation verifying the accuracy of the translation.

Statement

I, **Giovanna Luisa Sarolo**, hereby declare that I can read the Italian and English languages, and I am the translator that prepared the attached English translation of Italian patent application no. MI2003 A 001156, filed June 9, 2003, entitled "Mutations in the SLC40A1 Gene Associated to Impaired Iron Homeostasis." I certify to the best of my knowledge and belief that the attached translation is a true, complete and accurate English translation of Italian patent application no. MI2003 A 001156.

on January, 21 2009

Signature: ______________________

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LAI-2995830v1

SEAL

MINISTRY OF PRODUCTIVE ACTIVITIES
GENERAL DIRECTORATE FOR PRODUCTION DEVELOPMENT AND COMPETITIVITY
Italian Patent and Trademark Office
OFFICE G2

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Authentication of copy of documents concerning the Patent application for INDUSTRIAL INVENTION No. MI2003A001156 of 09 june 2003

It is hereby certified that the attached copy is the true copy of the original documents filed with the above mentioned patent application whose data are shown in the enclosed filing certificate

Rome,

Director of the Division
(signature)
Giampietro Carlotto

SEAL

FORM A
DUTY STAMP

TO THE MINISTRY OF INDUSTRY COMMERCE AND HANDICRAFT
Italian Patent and Trademark Office - ROME
Patent Application for Industrial Invention, filing of reserves, advanced opening to public inspection

A. Applicant
1) Name Antonello PIETRANGELO
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2) Name
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C. ELECTED DOMICILE OF THE ADDRESSEE
Address No. city code prov

D. TITLE proposed class, (sec./cl./ucl.) C120 group/subgroup 1/68

MUTATIONS IN THE SLC40A1 GENE ASSOCIATED TO IMPAIRED HOMEOSTASIS

ADVANCED OPENING TO PUBLIC INSPECTION yes___ no _X
in presence of amendment request: date no. of ref.:

E. NAMED INVENTORS

surname, name	surname, name
1) PIETRANGELO Antonello	3)
2)	4)

F. PRIORITY

Country or Exhibition Type of Priority Appln. No. Appln. date Encl(yes/res)
1) NONE
2)

G. CENTRE FOR COLLECTING MICROORGANISMS'CULTURES, denomination

H. SPECIAL NOTES
NONE

ENCLOSED DOCUMENTS No. Doc.		RESERVES DISSOLUTION Date	Filing. No.
Doc.1) 1 prov. no. sheets 74	abstract with main drawing, spec. and claims (compulsory 1 copy)		
Doc.2) 1 prov. No. Draw. 03	drawings (compulsory if cited in the description, 1 copy)		
Doc.3) 0 res. X	power of attorney or reference to general power of attorney		
Doc.4) 0 res.	designation of inventor		
Doc.5) 0 res.	priority doc. with Italian transl	Comparison single prio.	
Doc.6) 0 res.	authorisation or assignment deed		
Doc.7) 0 res.	complete name of the applicant		

8) PAYMENT RECEIPT OF EUR 472,56 compulsory

filled in on 09 June 2003 The applicant's signature Gemma Gervasi follows yes/no NO
We require a certified copy of the present deed yes/no YES

PROVINCIAL OFFICE OF INDUSTRY COMMERCE HANDICRAFT OF MILAN code 15

FILING CERTIFICATE Application no. MI2003A001156 Reg. A

The year 2003, the day 09 of the month of June

The above mentioned applicant(s) has(have) presented to me undersigned the present application consisting of no. 00 additional sheets for the grant of the above patent.

I. NOTES OF THE RECORDING OFFICER The Representative aware of the contents of Official Letter No. 423 dated 01 march 2001, files the application with reserve of the power of attorney

THE DEPOSITER
(signature)

THE RECORDING OFFICER
(signature)
Cortonesi Maurizio

FORM A

ABSTRACT OF THE INVENTION TOGETHER WITH MAIN DRAWING, DESCRIPTION AND CLAIM

Application No. MI2003A001156 Reg.A   Filing date 09 June 2003
Patent No.   Date of grant

D. TITLE

MUTATIONS IN THE SLC40A1 GENE ASSOCIATED TO IMPAIRED HOMEOSTASIS

L. ABSTRACT

The present invention relates to mutations in the SLC40A1 gene coding for the ferroportin 1, associated to impaired iron homeostasis or to non-HFE Hereditary Hemochromatosis and to methods for the diagnosis of these hereditary diseases based on the identification of said mutations.

M. DRAWING

Description of the Patent Application for the industrial invention entitled:

MUTATIONS IN THE SLC40A1 GENE ASSOCIATED TO IMPAIRED HOMEOSTASIS

Applicant: PIETRANGELO Antonello

Residing in Modena

named inventor: PIETRANGELO Antonello

filed on 09 June 2003 under No. MI2003A001156

* * * * *

FIELD OF INVENTION

The present invention relates to new mutations of the gene coding for Ferroportin 1 associated to a new variant of a genetic disorder characterized by iron accumulation and the identification of said mutations as a diagnostic method for Hereditary Hemochromatosis.

BACKGROUND OF THE INVENTION

Hemochromatosis is a genetic disorder characterized by an excess of iron accumulation in the body, causing in the course of the time injuries in different organs and tissues, particularly in liver, myocardium, pancreas, kidney, spleen, gonads and skin. Idiopatic Hemochromatosis is the most wide-spread hereditary disease in the Western population (incidence 1:300) and it is characterized by a recessive inheritance. This kind of Hemochromatosis was at first associated to HFE gene mutations (Hereditary Hemochromatosis described in Feder et al., Nat. Genet.1996, 13:399-408) . More recent studies have at first supposed and then proved that mainly in South-Western population, other genes in addition to HFE could have a role in Idiopatic Hemochromatosis (Piperno et al, Gastroenterology 1998,114: 996-1002 and Borot et al, Immunogenetics 1997, 45: 320-324).

Some mutations in the ferroportin gene, recently named SLC40A1 and previously known as SLC11A3 or IREG-1 or MTP-1, have indeed already been identified both by the authors of the present invention and by others as described for instance in

Montosi et al., J.Clin.Invest., 2001, 108:619 and in WO 02/033119; Devalia V. et al., Blood, 2002,100:695; Cazzola et al., British Journal of Hematology 2002, 119:539; Wallace et al., Blood, 2002, 100:692; Njajou Nat. Genet. 2001, 28:213.

The identification of most of the genetic alterations responsible for Hereditary Hemochromatosis or disases linked to impaired iron homeostasis is of great importance both in diagnostics and therapeutics. In fact, till today the diagnosis of Hemochromatosis is delayed and it is based on clinical symptomatology developed as a consequence of tissue injuries which are frequently irreversible. Moreover the diagnosis of such disease is made difficult by the fact that its sintoms are often similiar to those of other diseases characterized by impaired iron homeostasis.

The development of methods of genetic screening for the early diagnosis in a presynthomatic stage, of the Hereditary Hemochromatosis would allow to operate in time by phlebotomy to prevent in this way damages to organs and tissues.

Moreover the identification of genetic alterations linked to Hereditary Hemochromatosis and the comprehension of their role in the development of the pathology, are most relevant for the optimization of new and improved therapeutic strategies.

SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides coding for a ferroportin 1 which is mutated in at least one of the positions corresponding to the following aminoacids: position 80, position 174 or position 248 of IDN2 sequence. The identification of said mutations in the protein or in the nucleic acids coding for the protein is extremely useful for the diagnosis and therapy of non-HFE Hemochromatosis, Bantu Siderosis or African Hemochromatosis or for the predisposition to said diseases.

In addition the invention also relates to methods for the molecular diagnosis based on the use of oligonucleotides derived from said sequences or on the use of specific antibodies for said mutations.

Furthermore the invention also includes diagnostic kits for the identification of said

polymorphisms.

DESCRIPTION OF THE DRAWINGS

Fig.1:G80 mutation. Results of the diagnostic analysis of Hemochromatosis affected or non-affected family members.

Panel A shows the relationship among tested subjects (pedigree) in the family carring the G80 mutation. The subjects affected by Hemochromatosis are indicated in black, whereas the healthy ones are indicated in white. Circles indicate female subjects whereas squares indicate male subjects.

In panel B is visualized the electropherogram obtained by DNA automatic sequencer on the DNA fragment amplified according to the invention from a control (not carring the polymorphism) and from affected subject (carring the polymorphism).

Panel C showes the restriction patterns obtained by enzymatic cleavage with TspR1 of genomic DNA amplified from each subject by the sequencing primers IDN13 and IDN 14.

As shown in panel B, in healthy subjects carrying only the wilde type sequence after digestion with TspR1 the amplified DNA of 421 base pairs is not cleaved. In the subjects affected by the disease heterozygous for the mutation, the amplified DNA is digested into a band of 421 base pairs (wild type allele) and two fragments of respectively 238 and 183 base pairs (the latter is not visible in Fig. 2b). (+/+): homozygous subjects for wild type ferroportin, (+/-): heterozygous subjects for the mutation.

Fig. 2. N174 mutation. Results of the diagnostic analysis of hemochromatosis affected or non-affected family members.

Panel A shows the relationship among tested subjects (pedigree) in the family carrying the N174 mutation. Subjects affected by Hemochromatosis are indicated in black, whereas the healthy ones are indicated in white. Circles indicate female subjects whereas squares indicate male subjects.

In panel B is visualized the electropherogram obtained by DNA automatic

sequencer on the DNA fragment amplified according to the invention from a control (not carrying the polymorphism) and from affected subject (carrying the polymorphism).

Panel C showes the restriction patterns obtained by cleavage with BsmI of genomic DNA amplified by the sequencing primers IDN19 and IDN 20 from healthy and affected subjects.

In healthy subjects carrying only the wild type sequence, the amplified DNA of 425 base pairs is digested with BsmI into fragments of respectively 342 and 83 base pairs. In the subjects affected by the disease the polymorfism removes the enzyme target site and as a consequence the amplified DNA is not digested.

As the carrier individuals are heterozygous for the mutation, three different fragments will be obtained by BsmI digestion: a band of 425 bp (mutated allele) and two bands of 342 and 83 base pairs (wild type allele) respectively.

(+/+): homozygous subjects for wild type ferroportin, (+/-): heterozygous subjects for the mutation.

Fig. 3. Q248 mutation. Results of the diagnostic analysis of Bantu Siderosis affected or non-affected family members.

Panel A shows the portion of DNA sequence of exon 6 where it was detected the mutation in African Siderosis subjects and Black Americans.

Figure3b shows the restriction pattern obtained by PvuII digestion of the amplified DNA from different subjects with the primers of sequence IDN 19 and IDN 20. As shown in Figure 3b in healthy subjects carrying only the wild type sequence the amplified DNA of 425 base pairs is digested with PvuII restriction enzyme.

The mutation removes the enzyme cleavage site and only one out of two alleles is digested in a heterozygous subject for the mutation so that three bands will be obtained: a 425 bp band (mutated allele) and two bands of 302 and 123 base pairs (wild type allele) respectively.

(+/+): homozygous subjects for wild type ferroportin, (+/-): heterozygous subjects for

the mutation.

DETAILED DESCRIPTION OF THE INVENTION

The authors of the present invention have identified new mutations in the SLC40A1 gene (Solute Carrier Family) coding for ferroportin 1 (IREG1 or MTP1), previously also named SLC11A3, genetically linked to Hereditary Hemochromatosis or to an impaired non- HFE iron homeostasis (Hereditary Hemochromatosis).

The mutations described in the present invention were detected in the SLC40A1 gene coding for ferroportin, in the codons corresponding to aminoacids G80, N174 and Q248 of ferroportin1, where said notation is referred to the wilde type sequence with accession number NM_014585 (GenBank) and reported in the sequencing listing annex with the identification number 1 (seqIDN1,wild type). At genomic level the mutations are located in the exon 3 (G80 mutation) and in the exon 6 (mutations N174 and Q248) of the SLC40A1.

Said mutations cause aminoacid substitutions in the corresponding protein whose expression as a mutated form causes abnormal iron overload in carrier subjects.

From the functional point of view indeed ferroportin has a key role in at least two different but correlated aspects of iron homeostasis: in the enterocytes ferroportin causes the uptake of iron introduced by diet, whereas in the reticular endothelial cells particularly in macrophages, it causes the iron release from intracellular stores. Said new mutations are responsable for the Hemochromatosis and are characterized by clinical traits at least partially similiar to those already described in Pietrangelo et al. New England Journal of Medicine 1999, 341 (10): 725-732, caused by A77D mutation described in WO 02/033119.

Therefore, a first aspect of the invention refers to polymorphic polynucleotides related to SLC40A1 sequence, which encode for mutated forms of the wild type ferroportin 1 and concerning at least one of the following polymorphisms:

- polymorphism of the nucleotide corresponding to the nucleotide 238 of the IDN 1 sequence, preferably related to the substitution of a Guanine

with an Adenosine (G→A), which causes the replacement of aminoacid 80 with an aminoacid different from Glycine and preferably with Serine (G80S) in the coded protein: the cDNA derived from such polymorphic gene has preferably the IDN3 sequence;

- polymorphism of the nucleotide corresponding to the nucleotide 521 of the IDN 1 sequence, preferably related to the substitution of an Adenine with a Tymine (A→T), which causes the replacement of aminoacid 174 with an aminoacid different from Asparagine and preferably with Isoleucine (N174I) in the coded protein: the cDNA derived from such polymorphic gene has preferebly the IDN5 sequence;
- polymorphism of the nucleotide corresponding to the nucleotide 744 of the IDN1 sequence, preferably related to the substitution of a Guanine with a Tymine (G→T), which causes the replacement of amino acid 248 with an amino acid different from Glutamine and preferably with Hystidine (Q248H) in the coded protein: the cDNA derived from such polymorphic gene has preferably the IDN7 sequence;

or their oligonucleotide fragments of at least 10 base pairs.

The isolated polynucleotides obtained according to the invention and referred to the notation of the wilde type cDNA sequence with GenBank accession number N° NM_0145585, as partially reported in sequence IDN1, include at least one of the following substitutions:Guanine at position 552, preferably with Adenine, Adenine at position 835 preferably with Tymine, Guanine at position 1058 preferably with Tymine: this notation is referred to the aforementioned sequence in GenBank.

The oligonucleotides of the invention can be synthetized by chemical or enzymatic methods, or by enzyme digestion of isolated polynucleotides with restriction enzymes.

A preferred embodiment of the polynucleotides are the sequences IDN3,IDN5 and IDN7 or their fragments of at least 10 nucleotides and carrying at least one

of the aforementioned polymorphic substitutions wherein the sequences correspond to the cDNA coding for each of the mutated ferroportin sequences above described. When the polynucleotide is DNA it can be both single stranded or double stranded DNA, preferably the oligonucleotide is single stranded. Polynucleotides or oligonucleotides according to the invention can include modified nucleotides such as for example the thioderivatives nucleotides.

The invention also includes polynucleotides and oligonucleotides with complementary sequences to polynucleotides and oligonucleotides described in the invention and characterized in that they comprise the complementary nucleotide to at least one of the above described polymorphic nucleotides.

Preferably they are complementary to IDN1, 3 and 5 or their fragments as well as the oligonucleotides of at least 10 base pairs including at least one of the polimorphisms: then, preferably including the nucleotide complementary to the polymorphism at position 238 of IDN1 sequence, or the nucleotide complementary to the polymorphism of the nucleotide at position 521 of IDN1 sequence, or the nucleotide complementary to the polymorphism at position 744 of IDN1 sequence.

The polynucleotides and oligonucleotides complementary to the above ferroportin sequence can be used to specifically regulate the expression of the corresponding transcripts o can be used as specific probes to detect the presence of at least one of the aforementioned polymorphisms.

The oligonucleotides and polynucleotides of the invention can also be only partially identical or partially complementary to ferroportin 1 sequences identified as IDN 3, 5, 7 sequences or their fragments and so including non-homologous or non-identical regions. The complementary or homologous region to ferroportin or to its complementary sequence is in this case of at least 10 nucleotides. In particular it is fundamental that the addition of the nucleotides at 5' end or 3' end to the oligonucleotides do not affect the specificity in the

detection of the polymorphisms.

Complementary sequences can hybridize to each other under stringent conditions in a specific way. Consequently complementary polynucleotides and oligonucleotides of the invention can specifically hybridize to polynucleotides or to sequences carrying the mutations in the polymorphic sites, in particular to IDN3, 5 or 7 sequences and their fragments or oligonucleotides.

Furthermore the present invention includes oligonucleotides used for the amplification of genomic DNA regions or cDNAs comprisng the said mutations, thereof a preferred embodiment are oligonucleotides of IDN9-22 sequence, used to amplify as pairs genomic DNA of exon regions from 1 to 7 (for example sequencing primers IDN9 and IDN 10 amplify the exon 1, seq IDN 11 and 12 amplify the exon 2 and so on as described in more details in the experimental examples). Particularly preferred are oligonucleotides pairs of sequences IDN 13 and IDN 14 which amplify the exon 3 of the genomic DNA, including the polymorphism corresponding to the nucleotide 238 of the sequence IDN1 and IDN19 and IDN20 oligonucleotides pairs which amplify the exon 6 region, including the polymorphisms corresponding to nucleotides 521 and 744 of the sequence IDN1.

According to the present invention "nucleotide fragment" or polynucleotide refers to a nucleic acid with a partial sequence as compared to the sequences IDN 3, 5 and 7, longer than 50 nucleotides and including at least one of the aforementioned mutations or polymorphisms.

According to the present invention " oligonucleotide" refers to a nucleic acid with a portion of sequence as compared to sequences IDN 3, 5 and 7 with at least 10 bp.

According to a further and relevant aspect, the invention also refers to a protein, the ferroportin 1, essentially as an isolated and purified form, having a mutated aminoacidic sequence as compared to the wild type respectively at position corresponding to Glycine 80, or at position corresponding to Asparagine 174 or

at position corresponding to Glutamine 248, referred to the aminoacid sequence from the cDNA with accession number NM_014585 (GenBank).

The amino acids notation along the protein have the only purpose to unequivocally identify them, as they can change for example because of the presence of other species-specific mutations or for the presence of insertions or deletions in the DNA region coding for sequences upstream of said amino acid.

The G80S mutation casuses the substitution of Glycine, an amino acid of MW 75 with an intermediate polarity into Serine, an hydrophilic amino acid with MW 105. The N174I mutation causes the substitution of Asparagine into an uncharged hydrophilic amino acid of MW 132 into Isoleucine an uncharged hydrophobic amino acid of MW 131. The substitution of the amino acid 174 is of great importance for the protein as it is a glycosilation site. In addition the mutation at the position corresponding to the amino acid 248 of ferroportin 1 is a marker of the African variant of Hereditary Hemochromatosis, named African Siderosis, geographically localized in the Sub-Saharian regions and characterized by an excess iron deposition mainly in the reticular endothelial system, with an increase of early ferritinemia and only sometimes associated to a complete saturation of circulating transferrin.

These tracts are surprisedly similiar to the ferroportin-associated disease described (Pietrangelo et al. New England Journal of Medicin 1999, 341(10):725-732).

Some clinical traits associated to said mutation are indicated as follows:

i) in the G80S mutation carriers: ferritinemia increases at 1000-2000 ng/ml in untreated males; whereas in females ferritin usually do not exceed 700 ng/ml also in helderly females in a post-menopause age;

ii) in the N174I mutation carriers: it is observable a relevant increase of ferritinemia exceeding 4000 ng/ml also in females. It is likely that the mutation has a more severe effect on the structure and the function of the protein as compared to other mutations.

iii) in the Q248H mutation carriers: it is observable in Black Americans and Africans. Said mutation has an aggravating effect on a pre-existing iron overload condition. In American patients carriers for thalassemia , it causes a more severe phenotype with hyperferritinemia and iron deposition in reticular endothelial cells (macrophages) of liver and bone marrow, a typical trait of the disease as described by the authors of the present invention (Pietrangelo et al 1999) although patients were not subjected to blood transfusion (practice which can cause iron overload in macrophages). In African patients affected by Bantu Siderosis (that is associated to the excessive use of beer produced in iron containers) it is responsable for higher ferritinemia as compared to that found in patients which do not carry the mutation but drink comparable quantity of alcohol.

Paradoxically, the presence of the mutation also causes anemia, with a dramatic decrease of hemoglobin.

Furthermore the mutation is a marker of Black African population but it is not present in any Caucasic healthy donors, as demonstrated in parallel by the fact that the mutation was present in 6 out of 100 chromosomes of African individuals with the normal phenotype; similarly the mutation was found in four out of 100 Black Americans anonymous donors.

The analysis of these phenotipically healthy individuals showed a trend towards higher levels of ferritinemia and significantly lower hemoglobinemia as compared to non-mutated individuals. Therefore, the mutation in association with other factors (for example thalassemia and alcohol consuption) is responsible for a more severe phenotype. In addition in Black African and American populations it might have an effect in causing potentially lower levels of hemoglobin and potentially higher levels of ferritinemia as described more in details in the annotations of Table 1 in the Experimental Examples.

It is however to consider that hemoglobin and ferritinemia values are not by themselves sufficient to provide per se a diagnostic indication of non-HFE

Hemochromatosis, but only toghether with the presence of at least one of the mutations described in the invention. Such values can differ considerably from the above reported data, because of the presence of other factors such as the age of the subject or the time the diagnosis is carried out.

According to a further aspect, the invention comprises peptides or polypeptides longer than 5 amino acids with a portion of sequence corresponding to ferroportin1 protein sequence and characterized by the presence of mutations in the amino acid positions corresponding to Glycine at position 80, to Asparagine at position 174 or to Glutamine at position 248. Such peptides or polypeptides are obtained by chemical synthesis or by recombinant techniques. Preferably, polypeptides carrying at least one of the above identified mutations longer than 100 amino acids, are obtained by recombinant DNA techniques, whereas peptides including at least one of the above identified mutations, shorter than 100 amino acids are preferably obtained by chemical synthesis.

According to the structural prediction described in Davalia et al., G80 and N174 mutations are localized in the ferroportin extracellular domains, whereas the Q248 mutation is the first mutation mapping into an intracellular domain corresponding to amino acids 221-306, according to this prediction.

The domain carrying such mutation is then a further subject of the invention as for the first time it is surprisedly associated to polymorphisms causing clinical traits similar to those described for non-HFE Hereditary Haemochromatosis and able to cause a more severe phenotype when assiciated to other factors (for example alcohol consumption, Thalassaemia). Obviously the impairement of ferroportin functionality as a consequence of Q248 mutation, is not linked to the assignement to an intra or extracellular domain according to the secondary or tertiary structure prediction model and it is then not related to the strenght of the prediction model.

A further aspect of the invention relates to peptides whose sequence derives from seq IDN 2 (or 4 or 6 or 8) with a lenght of at least 5 amino acids and

including the corresponding amino acid at position 80, 174 and 248 of seq IDN 2 (or 4 or 6 or 8) and the amino acid just upstream or downstream said amino acids. The lenght and the sequence of said peptides are selected according to criteria known to the person skilled in the art on the basis of the preferred application.

A preferred embodiment of such peptides are the peptides corresponding to: Ile-Ile-X-Asp-Trp(G80) where X is different from Glycine and is preferably Serine; Asn-Met-X-Ala-Thr, where X is different from Asparagine and is preferably Isoleucine;Leu-Lys-X-Leu-Asn, where X is different from Glutamine and is preferably Hystidine; polypeptides comprising said peptides are also included in the present invention.They are useful for example to detect the presence of one of the described mutation by competition assays in cells or proteins, such as for example a cell extract or in diagnostic immonoassays.

Said peptides may carry at an N or C terminus amino acids residues non derived from ferroportin sequence and having a different function, for example "tag" peptides to facilitate the purification step.

By convention and according to the present invention, the term "fragment of polypeptide of the ferroportin protein" refers to a molecule corresponding to a partial sequence of mutated ferroportin 1 as described and carrying at least one of said mutations and having a sequence longer than 50 amino acids.

According to the invention the term peptide refers to a molecule whose sequence is a portion of the sequence of the mutated ferroportin 1, and carrying at least one of the said mutation with a lenght of at least 4 amino acids but shorter or equal to fifty amino acids. The present invention also comprises antibodies able to recognize in a specific way, as compared to the wild type protein, at least one of the G80, N174, Q248 mutations.

Such specific antibodies have a diagnostic application since the presence of the ferroportin carrying at least one of the said mutations is an early diagnostic marker of inherited impaired iron homeostasis disease.

Given the high incidence of non-HFE Hereditary Hemochromatosis (64% of the Italian Hemochromatosis variants) and in the rest of the world where have been described cases in Caucasian, Asiatic and other populations, and its continuous progression, polynucleotides, oligonucleotides, polypeptides or peptides, mutated ferroportin forms including said mutations as well as specific antibodies for the mutations identified in the protein, have an evident application in pharmaceutical, diagnostic and therapeutic areas.

In the diagnostic field nucleotides and polypeptides products of the invention are relevant for the diagnosis of non-HFE Hereditary Hemochromatosis, preferably for African and North American Hemochromatosis, for differential diagnosis of the hereditary or congenital hyperferritinemia, or for the diagnosis of anemia of unknown origin in young women or hyperferritinemia of unknown origin in child and adults.

Particularly in Bantu Hemochromatosis or African Siderosis the diagnosis of Q248 polymorphism is mainly useful to identify the genetic background of a more severe phenotype or the risk to develop phenotype together with other factors (alcohol consumption or Thalassaemia). The Q248 mutation is of great importance to identify the genetic background of an impaired iron homeostasis that in the individuals carrying the polymorphism is associated with a normal level of ferritinemia but with impaired levels of hemoglobinemia.

In vitro molecular diagnosis, based on the identification of DNA or protein mutation as described in the present invention, and carried out by methods and reagents described in the present invention, allows the early diagnosis of Hereditary Hemochromatosis.

Early diagnosis is necessary for this disease which is asynthomatic until the individual is 30 years old, and which is frequently diagnosed only after the appearance of adverse effects caused by iron accumulation in the involved organs (lung, liver, joints, pancreas) when their function is already irreversibly demaged.

Oligonucleotides and polynucleotides including the polymorphism causing the Q248 mutation are also useful as genetic marker for Black African population and are used for the study of the genetic linkage for those disease whose defective genes map on the same chromosome.

Nucleic acids of the invention are useful in the therapeutic area particularly in substitutive genetic therapy, where by homologous recombination with wild type sequences and/or for cell therapy they are the target of said sequences.

In fact as the presence in an individual of the gene carrying at least one of the mutations of the invention and the corresponding product (mutated ferroportin 1) is correlated with the outbreak of Hereditary Hemochromatosis, it is of great importance to have the instruments to knock out the expression of the gene or to inactivate the protein. The invention refers then to pharmaceutical compositions including said oligonucleotides, antibodies or peptides mixed with pharmaceutically acceptable excipients.

In one of the most common applications the nucleic acids of the invention, preferably the oligonucleotides shorter than 50 bp, preferably of at least 40 bp or more preferably with the lenght between 8 and 25, or 8 and 15 nucleotides are used to assay the presence of said polymorphisms in a biological sample.

However the present invention also refers to the therapeutic use of polynucleotides and oligonucleotides of the invention. Typically said oligonucleotides include the aforementioned polymorphism or they have complementary sequence to the region comprising said polymorphism and they are therefore allele specific oligonucleotides and polynucleotides.

Preferably oligonucleotides or nucleic acids or the invention include the following decamers or the corresponding complementary sequences: 5' ATCAGTGACT 3' (seq IDN 23) including the underlined polymorphism and responsible for the G80S mutation, 5' GATGATTGCC 3' (seq IDN 24) including underlined polymorphism and responsible for the mutation N174I, 5' GAAACATCTG 3' (seq IDN 25) including underlined polymorphism and responsible for the mutation

Q248H. Therefore they can include additonal nucleotides at 5' and at 3' ends only if these do not affect the binding specificity, for example by hybridization to a ferroportin sequence , for the polymorphisms whose therapeutic and diagnostic importance is herein described as a subject of the present invention.

Polynucleotides of the invention, particularly IDN3,5,7 or their fragments can also be used for the production of recombinant ferroportin 1 molecules or chimeric proteins or truncated forms of the protein including at least one of the mutated amino acids at position G80, N174, Q248. They are inserted into expression vectors and used to transform prokaryotic and eukaryotic cells accoding to art-known techniques such as for example, transfection, transformation, infection or intranuclear injection. Vectors suitable to this aim include, for example, plasmids, viral vectors and yeast or mammalian artificial chromosomes.

According to a further application, the invention refers therefore to a recombinant vector carrying a nucleic acid or a DNA fragment according to the invention as well as to eukaryotic or prokaryotic cells transformed with said vectors. The person skilled in the art is able to choose each time fragment and oligonucleotides with sequences and lenght suitable to the preferred use. For example, if the fragments or oligonucleotides are used for the identification of a mutation described in the invention by hybridization techniques their lenght and sequence is choosen to get a specific hybridization under stringent conditions to a nucleic acid sequence including the mutated codon.

Allele-specific oligonucleotide probes are longer than 10 nucleotides, preferably between 15 and 50 nucleotides and more preferably not longer than 35 nucleotides, preferably between 15 and 30 nucleotides in lenght. The sequence of such probes is choosen by the person skilled in the art who select them on the basis of the full lenght sequence also by the use of known softwares and according to the assay they will be used in. Preferably they include at least one of the oligonucleotide sequences IDN23,IDN24 or IDN25 which are

characterized by the fact to include the polymorphisms of the nucleotide 238, 521 and 744 respectively, according to the sequence notation IDN1.

The fragments and oligonucleotides of the invention can be labelled, for example with one or more markers choosen among radioisotopes, enzymes, biotine-avidine or other fluorescent molecules able to detect them by specific assays.

According to a further aspect the invention relates to oligonucleotides and polynucleotides characterized for comprising the above described polymorphisms and nucleic acids complementary to them, as well as peptides and proteins corresponding to the mutated ferroportin form for therapeutic use.

Then, due to the importance and incidence of Hereditary Hemochromatosis the invention includes all the nucleic acids and proteins of the invention for therapeutic use. According to a preferred aspect the invention relates to nucleic acids with sequence IDN 3, 5 and 7 and their fragments, the oligonucleotides with the sequences IDN 23-25 and those complementary to them, proteins with sequences IDN 4, 6, 8 and their derived peptides including the amino acid substitution derived from the polymorphism, for therapeutic use.

The polynucleotides according to the invention can also be used for cells and non-human transgenic mammals preparations including the transgene coding for at least one of the mutated forms of ferroportin 1 of the invention. The transgene can be stably inserted in the genome of the animal cell or it can be present as a transient form.

Said non human cells, tissues or animals are useful as models for the study of gene and protein function including the mutations according to the invention and of their role in the outbreak of the Hereditary Hemochromatosis. These models are particularly important for the development of new therapeutic approaches for the treatment of non HFE-Hereditary Hemochromatosis or of the impaired iron overload homeostasis

In a further aspect the invention refers to a method for in vitro diagnosis of non

HFE-Hereditary Hemochromatosis, or African Siderosis or Bantu Hemochromatosis in a mammal, preferably Homo Sapiens also in cases where the only detectable clinical trait is only hyperferritinemia or anemia and including the following steps:

a) isolation of nucleic acids contained in a biological sample obtained by said mammal;
b) Test for the presence of a mutation or a polymorphism according to the invention in said nucleic acid,

where the presence of at least one of said mutation or polymorphisms consists of an indication that said mammal is affected by an hereditary defect in the regulation of iron homeostasis, or more specifically affected by non-HFE Hereditary Hemochromatosis, African Siderosis, hereditary anemia with hyperferritinemia or hereditary disease associated to iron overload in reticular endothelial cells.

Preferably said biological sample is a sample of plasma, saliva, urine, faeces, amniotic liquid or tissue or it consists of cells from biopsies. Preferably said nucleic acid is genomic DNA or RNA. If the nucleic acid is RNA it is preferably transformed into complementary DNA (cDNA) by a reverse transcription reaction.

Genomic DNA or cDNA are directly analyzed or after in vitro amplification by polymerase chain reaction (PCR) (Saiki et al., Science 239:487-491, 1988) or other techniques such as, for example, ligase chain reaction (LCR) (Wu et al.,Genomics 4:560-569, 1989) strand displacement amplification (SDA) (Walker et al., PNAs USA 89:392-396) or self-sustained sequence replication (3SR) (Fahy et al., PCR Methods Appl 1: 25-33, 1992). Preferably genomic DNA or cDNA is amplified by PCR using a pair of oligonucleotides suitable for the amplification of the DNA fragment including the codon coding for the amino acid corresponding to position 80 or 174 or 248 of seq IDN 2.

Oligonucleotide pairs suitable for the amplification of the region containing the mutation G80 on genomic DNA, can also amplify exon 3 whose sequence corresponds to sequences IDN13 and IDN 14, whereas Oligonucleotides suitable for the amplification of the region including N174 and Q248 mutations on the exon 6 refers to sequences IDN19 and IDN 20. Oligonucleotides of sequence IDN 9-22 are therefore comprised in the present invention. Particularly preferred is the oligonucleotides pair that amplify the region of the exon 3 comprising the polymorphism responsible for the G80 mutation that is the pairs consisting of IDN13 and IDN 14 and the oligonucleotides pairs that amplify the region of the exon 6 including the polymorphism responsible for the Q248 mutation and the polymorphism responsable for N174 mutation such as the oligonucleotides pairs consisting of sequences IDN19 and IDN20. Oligonucleotides specific for the exon carrying the mutation can be identified on the genomic DNA sequence close to the sequences identified by said oligonucleotides. Therefore, the present invention comprises oligonucleotides with at least 8 nucleotides of each oligonucleotide with sequence IDN 9-22, preferably with sequence IDN13 and 14 and 19-20.

Several art-known techniques can be used to identify the presence of mutations according to the invention in genomic DNA or cDNA.

Proper techniques for example are based on the use of restriction enzymes (Kan et al, Lancet: 910-912, 1978), techniques of hybridization with allele-specific oligonucleotide probes (Wallace et al, Nucl Acids Res 6: 3543-3557, 1978) as for example hybridization with oligonucleotides immobilized on filters (Saiki et al, PNAS USA 86: 6230-6234, 1989) or micro-chips (Chee et al, Science 274:610-614, 1996) and *oligonucleotide arrays* (Maskos et al, Nucl Acids Res 21: 2269-2270, 1993), allele-specific PCR (Newton et al. Nucl Acid Res 17:2503-2516, 1989), *mismatch repair detection (MRD)* (Faham e Cox Genome Res: 474-482, 1995), *Single-strand conformational polymorphism analysis* (Ravnik-Glavac *et al*, Hum. Mol. Gen. 3: 801, 1994), gel electrophoresis

on denaturing gradient (Guldberg et al., Nucl. Acids Res. 22: 880, 1994), *Hot Cleavage* (Cotton et al. Proc.Natl. Acad Sci USA 85: 4397, 1988), *DNAse* (Youil et al, PNAS USA 92: 87-91, 1995) and *RNAse protection assay* (Winter *et al.* Proc. Natl. Acad. Sci. USA, 82: 7575, 1985; Meyers *et al.*, Science 230: 1242, 1985), *allele specific primer extension* (Syvanen et al, Genomics 8: 684-692, 1990 and Syvanen et al, Hum Mutat 13:1-10, 1999), *genetic bit analysis* (GBA) (Nikiforov et al Nucl Acid Res 22:4167-4175, 1994), *primer-ligation assay* (OLA) (Landergen et al, Science 241: 1077, 1988), *allele specific ligation chain reaction* (LCR) (Barrany PNAS USA 88:189-193, 1991), *gap-LCR* (Abravaya et al Nucl Acids Res 23: 675-682, 1995), sequencing techniques, or Ligase Detection Reaction (described in US 6,312,892).

Particularly preferred techniques for the identification of the mutation of the invention are based on the use of restriction enzymes that is presence only in presence of the aforementioned polymorphism, on allele-specific PCR, on hybridization, on direct sequencing or "computer readable" micro array.

Furthermore according to a preferred embodiment the control for the presence of the mutation according to the invention in the analyzed DNA, is performed by using techniques based on the use of restriction enzymes and comprising the following steps:

a) amplification of genomic DNA or cDNA with an oligonucleotides pairs suitable for the selective amplification of the fragment of said DNA comprising the codon coding for the amino acid corresponding to position G80 or N174 or Q248, where preferably such amplification occurs with oligonucleotides pairs 13 and 14 for the mutation on the exon 3 (G80) and the oligonucleotides pairs 19 and 20 for the mutation on the exon 6 (N174 and Q248);
b) incubation of the amplified DNA with a restriction enzyme able to recognize the restriction site modified (produced or removed) by the mutation.
c) analysis of the products size of said digestion and optionally comparison with

the restriction pattern obtained from a healthy donor; where the presence or the absence of enzymatic digestion in at least a chromosomic allele indicate the presence in the analyzed individuals of at least one of the mutations responsable for non-HFE Hereditary Hemochromatosis.

The analysis of the size of the products after digestion is performed for example by gel electrophoresis by the use of a molecular weight marker, followed by visualization of the DNA bands for example by ethidium bromide.

As it will be shown in details in the Experimental Examples describing one of the preferred embodiment of the diagnostic method of the invention, the polymorphism of the nucleotide at position 238 (G$\rightarrow$A) which causes the substitution G80S in the corresponding protein also generates the restriction site for the enzyme TspRI: the 421 bp fragment amplified with primers of sequence IDN13 and 14 (exon 3), is digested only when the polimorphism is present, in two bands of 238 and 183 bp, whereas it is not affected in the wild type. The presence of the polymorphism of the nucleotide 521 (A$\rightarrow$T) which causes the substitution N174I in the corresponding protein is detected after amplification of genomic DNA with the primers pairs corresponding to exon 6 (seq ADN 19 and 20), by digestion with BsmI. The polymorphism causes the loss of the recognizing sequence for the restriction site and therefor after DNA amplification and digestion the whole fragment of 425 bp is detected: in the normal individual (wild type), indeed the amplified DNA is digested into two fragments of 342 and 83 bp.

The presence of the polymorphism G$\rightarrow$T at position 744 of seq IDN1, causing the substitution Q248H in the corresponding protein, is detectable after amplification of the exon region of 425 bp with the primers pair of sequence IDN19 and 20 (exon 6) by digestion with PvuII: the mutated sequence removes the restriction site of the enzyme and a band of 425 bp is detected, whereas the presence of the wild type allele is detectable as a band of 305 bp and a band of 123 bp.

Aforementioned polymorphisms can be detected throughout the loss or gain of said restriction sites, by selecting suitable primers for the amplification also on the cDNA.

According to a further embodiment the identification of the mutation of the invention is performed by hybridization techniques where nucleic acid fragments of the invention or oligonucleotides specific for the mutation of the invention are used. Said fragments or oligonucleotides are able to specifically hybridize to a sequence of the nucleic acid of the invention including the mutated codon also when said sequence is present together with several other sequences.

The man skilled in the art is able to select each time the hybridization conditions and the length and the sequence of the fragments or of oligonucleotides more suitable to the specific hybridization technique used and to the kind of DNA under evaluation (genomic or complementary DNA amplified or cloned into suitable vectors).

The method to detect the polymorphisms described in the invention is of diagnostic importance to detect the genetic background of iron impaired homeostasis where such iron impaired homeostasis can bring to both anemia and hyperferritinemia. In particular Q248H polymorphismis is of diagnostic importance to detect the genetic background of the disease identified as African or Bantu Siderosis or of a simple anemia.

According to a further preferred embodiment the diagnostic method relates to the use of an allele-specific PCR where the genomic or complementary DNA is subjected to a PCR reaction where oligonucleotides able to selectively amplify a fragment of said DNA comprising the mutated codon but not the corresponding fragment carrying the non mutated codon are used.

In a particularly preferred embodiment the oligonucleotides of the invention used to detect the presence of at least one of the described polymorphisms of the invention are chemically fixed to a solid support preferably of glass or to microchips (bidimentional or spheric as the "beads"), which are "computer

readable", are preferably arranged as a matrix (array system) and are characterized by the fact to comprise at least one of the polymorphisms of the invention or at least one of the oligonucleotides or polynucleotides of the invention.

Furthermore the present invention comprises diagnostic kits for the diagnosis of the genetic background associated to an impaired iron homeostasis caused by the aforementioned polymorphisms, associated or not to hyperferritinemia or to anemia based on the DNA molecular analysis. Said kits are characterized by comprising at least one of the oligonucleotides or polynucleotides of the invention, detecting the polymorphisms subject of the present invention. According to particularly preferred embodiment said diagnostic kits comprise the oligonucleotides pairs for the amplification of exon 3 (seq IDN13 and 14) and the oligonucleotide pairs for the amplification of exon 6 (seq IDN19 and 20), and the enzymes Tspr1 and BsmI and PvuII. As an alternative said kits also include polynucleotides comprising oligonucleotides of sequence IDN25, 26 or 27. Moreover, said kits can possibly include also oligonucleotides and the restriction enzyme to detect the A77D mutation caused by the polymorphism described in the patent application WO 02/33119.

The present invention also refers to a method for the in vitro diagnosis of Hereditary Hemochromatosis in a mammal, including the evaluation of the presence in a biological sample of said mammal, the presence of a mutated ferroportin1 protein according to the invention, where the identification of said protein is an indication that the individual is affected by Hereditary Hemochromatosis.

Preferably said test is performed by immunological assays using monoclonal or polyclonal antibodies able to discriminate between a mutated ferroportin molecule according to the invention and a wild type ferroportin molecule.

Therefore, the present invention also refers to monoclonal and polyclonal antibodies able to specifically recognize a mutated ferroportin molecule

according to the invention or a peptide or an epitope comprising the mutation. Such antibodies are obtained by art-known techniques such as, for example, those methods described by Harlow and Lane in Antibodies, A Laboratory Manual, Cold Spring Harbour Laboratory, 1988.

Antibodies of the invention are particularly useful as diagnostic reagents but also to study protein features or for therapeutic approaches. For example, said antibodies can be used to detect the exact tissues or cells localization of the mutated protein, to study its biochemical characteristics or to purify it by immunoaffinity assay.

Therefore are comprised in the present invention kits for the study of the function of mutated ferroportin forms based on immunospecific identification of mutated ferroportin forms, preferably including antibodies specific for G80S, N174I, Q248H mutations and optionally peptides or mutated proteins standards expressed as recombinant products and optionally peptides able to compete with the ligand, for the setting up of ELISA assays or Western Blot, or radioimmunoprecipitation assays on fluid or solid phase.

EXPERIMENTAL EXAMPLES

EXAMPLE1. Identification of the mutations in the ferroportin gene.

Genomic DNA of index cases, of family members and of control subjects, was extracted from leucocytes obtained by blood samples using a blood DNA extraction kit (Quiagen).

Obtained DNA was then amplified by PCR using primers pair able to amplify the whole coding region including exon/intron boundary regions of the ferroportin.

Primers pairs used herein are the following:

Exon 1:	Fw.1: 5'-GGTGCTATCTCCAGTTCCTT-3'	(IDN 9)
	Rv.1: 5'-GTTCACAGCAGAGCCACATT-3'	(IDN 10)
Exon 2:	Fw.2: 5'-CAGCTCATTAAGTGACTACCATCGC-3'	(IDN 11)
	Rv.2: 5'-GGCTTAATACAACTGGCTAGAACG-3'	(IDN 12)
Exon 3:	Fw.3: 5'-CATAATGTAGCCAGGAAGTGCCC-3'	(IDN 13)
	Rv.3: 5'-TCCAGAGGTGGTGCCATCTAAG-3'	(IDN 14)
Exon 4:	Fw.4: 5'-GAGACATTTTGATGTAATGTACAC-3'	(IDN 15)
	Rv.4: 5'-CTACCAGATATTCAATTTTCTGCC-3'	(IDN 16)
Exon 5:	Fw.5: 5'-CCACCAAAGACTATTTTAAACTGC-3'	(IDN 17)
	Rv.5: 5'-TCACCACCGATTTAAAGTGAATCC-3'	(IDN 18)
Exon 6:	Fw.6: 5'-GTATTGTGTAAATGGGCAGTCTC-3'	(IDN 19)
	Rv.6: 5'-CCCCACTGGTAATAAAACCTG-3'	(IDN 20)
Exon 7:	Fw.7: 5'-GGCTTTTATTTCTACATGTCCTCC-3'	(IDN 21)
	Rv.7: 5'-ACATTTAGGGAACATTTCAGATC-3'	(IDN 22)
Exon 8:	Fw.8: 5'-AAGGTGACTTAAAGACAGTCAGGC-3'	(IDN 23)
	Rv.8: 5'-GCTGACTTAGGTTTCCTAAACAGC-3'	(IDN 24)

The amplification of the regions corresponding to each exon was performed as follows: 200 ng of genomic DNA were amplified in 50 μl of reaction buffer 1X containing dNTPs 200 μM, MgCl2 1,5 mM, 25 pmoles of each of the aforementioned oligonucleotides, 1 U of Taq polymerase (Applied Biosystems).

In the amplification reaction was used a program of 30 cycles, each characterized by the following thermal profile:

94°C for 1 minute,

58°C for 40 seconds,

75°C for 5 minutes.

Obtained fragments were purified and sequenced by automatic sequencing with the Backman Coulter Sequencer. The sequence analysis allowed the identification of

The G80S mutation in the exon 3 and the N174I and Q248H mutations in the exon 6, as compared to the wild type sequence (GenBank accession number: AF231121) that was not detected in any of the control subjects.

A further evaluation of the mutations was performed by the digestion of an aliquote of the same first PCR product with endonucleases whose restriction site is modified by the nucleotide substitution.

In particular, the Q248H mutation was verified by digestion according to the Manufacturer's Instructions (New England Biolabs), the first product of PCR with the PvuII enzyme , which cut into GC in the 5 'CAGCTG 3' sequence. The G→ T base substitution in the mutated sequence removes the restriction site of the enzyme.

EXAMPLE 2

Characterization of clnical features of the subjects carrying Q248H mutation

The clinical features of African normal control or Bantu Siderosis affected subjects (associated to excess beer consumption produced into iron containers) carrying the Q248H mutation was evaluated. In said subjects the mutation correlates with higher hyperferritinemia as compared to those subjects without the mutation, but drinking comparable quantity of alcohol.

Paradoxically the presence of said mutation also causes an anemia status with highly significant decrease of hemoglobin. Then, the mutation has an aggravating effect on a preexisting status of iron overload.

In Black American patients carriers for thalassaemia , the mutation causes a more severe phenotype with hyperferritinemia and iron deposition in reticular endothelial cells (macrophages) of liver and bone marrow, although patients were not subjected to blood transfusions (practice which can cause iron overload in macrophages). Hyperferritinemia and iron accumulation in reticular endothelial cells correspond to the clinical features observed by the same authors of the present

invention in Pietrangelo et al.;1999 N. Engl. J. Med 3341:725-732.

Moreover the mutation is a marker of Black African population: it resulted in fact absent in a sample of 300 healthy White Caucasian donors.

In African population 100 chromosomes from phenotipically normal African subjects were assayed and 6 out of such chromosomes carried the mutation.

Similarly, the mutation was found in 4 out of 100 of a group of Black American donors. The analysis of these phenotipically healthy subjects showed a trend towards higher levels of ferritinemia and significantly lower hemoglobinemia as compared to non-mutated individuals. Then the mutation is not able to cause a disease, but it is resposable for a more severe phenotype in association with other factors (for example thalassaemia and alcohol consuption). In addition in Black African and American populations it might have an effect in causing potentially lower hemoglobin levels and potentially higher ferritinemia levels. These conclusions also arise from Table1 of the Experimental Examples where are reported data concerning ferritinemia and hemoglobin in patients carrying the mutation in Africans, Americans and in phenotipically healthy Black populations.

Table 1 Evaluation of the “iron status” and hemoglobin levels in relation to the Q248H of the ferroportin in Africans and Afrio-Americans. The members of the families come from three African and one Afrio-American pedigrees.

The number of individuals in each group is indicated under each parameter (N=).

	Ferroportin Q248H mutation	**Ferroportin wild type**	**p**
Families Members (affected cases are not included)	**(N = 10)**	**(N = 11)**	
Ferritin (μg/L; mean and SE range)	76(47-125)	95(62-147)	0.748
Ferritin/AST ratio* (μg /U; mean ±SE)	14.7±4.6	5.5±4.1	0.171
Transferrin Saturation (%; mean ±SE)	36 ± 7	22 ± 8	0.258
Hemoglobin*** (g/dL; mean ± SE)	11.8 ± 0.6	13.3 ± 0.5	**0.088**
Normal Africans	(N = 7)	(N = 44)	
Ferritin (μg /L; mean and SE range)	61(38-97)	34(28-40)	0.251
Transferrin Saturation (%; mean ± SE)	28 ± 5	26 ± 2	0.684
Hemoglobin (g/dL; mean ± SE)	12.5 ± 0.5	13.7 ± 0.2	**0.039**
Families members and combined controls	**(N = 17)**	**(N = 55)**	
Ferritin/AST ratio (μg /U; mean ±SE range)	61(44-82)	44(37-51)	0.358
Transferrin Saturation (%; mean ± SE)	30 ± 4	26 ± 2	0.357
Hemoglobin (g/dL; mean ± SE)	12.1 ±0.4	13.6±0.2	**<0.0005**

Statistical analysis was performed by the ANOVA test adjusted for the age, gender and for Africans, for beer consumption. In the screening pilot study of Q248H mutation were included the family members of patients with iron overload (N=21) and African subjects with normal values of iron metabolism . It is evident in said "normal" population that the presence of Q248H mutation is associated to a trend to an increase of ferritin levels and particularly to a significant decrease of hemoglobin.

EXAMPLE 3 Set up of the diagnostic method by PCR

By the sequencing of exons regions amplified as described in the EXAMPLE1, it was evident that the polymorphism of 238 nucleoide of the IDN1 sequence, consisting on the substitution of a Guanine by an Adenine (G→ A) responsible of the substitution of the Glycine at position 80 of IDN2 sequence by a Serine (G80S) in the corresponding coded protein, causes the generation of a cleavage site for TspR1 enzyme.

The sequence of the full lenght cDNA coding for the mutated form of ferroportin at position 80 (G80S) is reported as IDN3 sequence in the sequences listing annex..

Figure 1B shows the restriction pattern of the amplified genomic DNA of each individual: in the healthy subjects havng only the wild type sequence, after digestion with Tspr1 the fragment of amplified DNA with the oligonucleotide pairs 13 1nd 14, of 421 base pairs, is not cleaved.

In affected subjects, heterozygous for the mutation, the amplfied DNA is cleaved into a band of 421base pairs (wild type allele) and two bands of 238 and 183 base pairs (this last not visible in FIGURE 1b).

The polymorphism of nucleotide 521 of IDN1 sequence, consisting in the substitution of an Adenine by a Tymine (A→ T), causing the substitution of Asparagine with an Isoleucine at position 174 (N174I) in the corresponding coded protein , whereas it causes the knock out of the cleavage site for the restriction

enzyme BsmI and as a consequence the DNA fragment of exon 6 from individuals carrying the polimorphisms amplified by oligonucleotide pairs 19 and 20 is not cleaved. The sequence of the full lenght cDNA coding for the mutated form of ferroportin at position 174 (N174I) is reported as IDN5 sequence in the sequencing listing annex. Figure 2 panel B shows the restriction pattern obtained after digestion with BsmI of amplified DNA from healthy individuals carrying the polymorphism. In case of healhy subjects having only the wild type sequence, after digestion with BsmI of the DNA fragment of 425 bp amplified with primer pairs 19 and 20, it is digested into two fragments of 342 and 83 base pairs. In carriers subjects, heterozygous for the mutation, after digestion with BsmI three bands were visualized: a band of 425 base pairs (mutated allele) and two bands of 342 and 83 base pairs (wild type allele).

The polimorphism of nucleotide 744 of IDN1 sequence, consisting on the substitution of a Guanine by a Tymine (G$\rightarrow$ T), causes the substitution of the aminoacid at position 248 (Glutamine) with Hystidine (Q248H) in the corresponding coded protein and the knock out of the cleavage site of PvuII enzyme. The sequence of the full lenght cDNA coding for the mutated form of ferroportin at position 248 (Q248H) is reported as IDN7 sequence in the sequences listing annex. In Figure 3B is reported the restriction pattern obtained by cleavage with PvuII enzyme of amplified DNA from healthy individuals or carriers of the polymorphism: in healthy subjects having only the wild type sequence, the amplfied DNA of 425 bp is cleaved by PvuII restriction enzyme. In hetrozygous carriers subjects, only one allele is cleaved, therefore obtaining three bands: a band of 425 bp (mutated allele) and two bands of 302 and 123 bp (wild type allele).

CLAIMS

1. Isolated polynucleotides coding for a ferroportin1 mutated in one of the following amino acids:
   - amino acid at position 80 of seq IDN2,
   - amino acid at position 174 of seqIDN2,
   - amino acid at position 248 of seqIDN2,

   as compared to the wild type sequence.

2. Polynucleotide according to claim 1 characterized in that the amino acid at position 80 is different from Glycine
3. Polynucleotide according to claim 2 characterized by having a polymorphism at nucleotide 238 of IDN1 sequence.
4. Polynucleotide according to claim 3 characterized in that said polymorphism is a substitution of a G with an A.
5. Polinucleotide according to claim 1 charachterized in that said amino acid at position 174 is different from Asparagine.
6. Polinucleotide according to claim 5 characterized by having a polymorphism at nucleotide 521 of IDN1 sequence.
7. Polynucleotide according to claim 6 characterized in that said polymorphism is a substitution of an A with a T.
8. Polynucleotide according to claim 1 characterized in that the amino acid at position 248 is different from Glutamine.
9. Polynucleotide according to claim 8 characterized by having a polymorphism at nucleotide 744 of IDN1 sequence.
10. Polynucleotide according to claim 9 characterized in that said polymorphism is a substitution of a G with a T
11. Polynucleotide according to claims 1-10 characterized in that it is genomic DNA.
12. Polynucleotide according to claims 1-10 characterized in that it is mRNA.
13. Polynucleotide according to claims 1-10 characterized in that it is cDNA.

14. Polynucleotides coding for a mutated ferroportin according to claim 1 characterized in that nucleotide sequence corresponds to seqIDN3 or to seqIDN5 or to seqIDN7.

15. Polynucleotide of at least 10 consecutive nucleotides derived from sequence IDN 3,5 or 7 and characterized by comprising at least one of polymorphic nucleotides respectively choosen among the following:
polymorphism corresponding to position 238 of seqIDN3,
polymophism corresponding to position 521 of seq IDN5, or
polymorphism corresponding to position 744 of seqIDN7.

16. Polynucleotides according to claim 15 characterized by comprising at least one of the oligonucleotides with sequence corresponding to sequences IDN 9-27.

17. Polynucleotides having a complementary sequence as compared to polynucleotides according to claims 14-16.

18. Polynucleotides according to claim 1-16 characterized in that it is labelled.

19. Recombinant vector characterized by comprising the polynucleotide according to claim 1-17.

20. Isolated cell characterized in that it is transfected or transformed with the recombinant vector according to claim 19

20. Eukaryotic cell, tissue or non-human animal including a transgene where such transgene is at least a polynucleotide according to claim 1-16.

21. Mutated ferroportin1 coded by polynucleotides according to claims 1-16.

22. Mutated ferroportin according to claim 21 having amino acid sequence corresponding to at least one of the sequences IDN 4, 6, 8 or their fragments.

23. Peptide having a lenght of at least 6 amino acids and a partial sequence derived from at least one of the sequences choosen among the following: seq IDN 4, 6 or 8 and characterized by comprising at least one of the mutated amino acids at position corresponding to positions 80, 174, 248 of seqIDN2.

24. Polynucleotides according to claims 1-17 for therapeutic use.

25. Peptides acoording to claim 23 for therapeutic use.

26. Mutated ferroportin 1 according to claim 22 for therapeutic use.

27. Method to detect polymorphisms in the ferroportin gene characterized by using at least one of the polynucleotides according to claim 1-18.

28. Method according to claim 24 where said polymorphisms are also associated to hyperferritinemia or anemia.

29. Method for the in vitro diagnosis of non-HFE Hereditary Hemochromatosis in a mammal including the following steps:

a) isolation of genomic DNA or RNA by a biological sample obtained from a mammal.

b) evaluation of the presence in said genomic DNA or RNA of at least one of the polymorphisms according to claim 4, 7, 10, where the presence of at least one of said polymorphisms is an indication that said mammal is affected by non-HFE Hereditary Hemochromatosis or is prone to develop said disease

27. Method for the in vitro diagnosis of an impaired iron homeostasis consisting essentially in the evaluation of the presence in a sample of genomic DNA , RNA or cDNA of at least one of the polimorphisms choosen among the following: polymorphism corresponding to position 238 of seq IDN3, polymorphism corresponding to position 521 of seq IDN5 or polymorphism corresponding to position 744 of seqIDN7.

28. Method according to claim 27 where such iron impaired homeostasis is anemia or hyperferritinemia, African or Bantu siderosis, or non-HFE Hereditary Hemochromatosis.

29. Method according to claim 28 for the in vitro diagnosis of African Siderosis or Bantu Hemochromatosis in a mammal including the following steps:

a) Isolation of genomic DNA or RNA from a biological sample obtained by said mammal.

b) evaluation of the presence in said genomic DNA or RNA of a polymorphism of the nucleotide corresponding to nucleotide 744 of seq IDN1.

where the presence of such polymorphism is an indication that said mammal is affected by African Siderosis, Bantu Haemochromatosis or is prone to develop said disease.

30. Method according to the claims 25-28 characterized in that the before said evaluation the RNA is transcribed into cDNA by reverse transcriptase.

31. Method according to the claims 26-30 where this evaluation is performed after the amplification by PCR with suitable oligonucleotide pairs , of a DNA fragment including at least one of the following polymorphic nucleotides:nucleotide corresponding to position 238, nucleotide corresponding to position 521, nucleotide corresponding to position 744 of seq IDN1.

32. Method according to claim 31 characterized in that in said amplification is used at least one of the following oligonucleotides: IDN13, 14, 19, 20.

33. Method according to claims 26-32 characterized in that said mammal is Homo Sapiens.

34. Method according to claims 25 and 28 characterized in that said biological sample is a sample of blood, plasma, saliva, urina, faeces, amniotic liquid or tissue.

35. Method according to the claims 25-34 characterized in that said evaluation is performed using a technique choosen in the list consisting of: gain or loss of a cleavage site for a restriction enzyme, hybridization techniques with allele-specific oligonucleotide probes according to the claims 15-17, allele specific PCR, mismatch repair detection, single strand conformational polymorphism analysis, gel electrophoresis on denaturing gradient, hot cleavage, DNAse and RNAse protection assay, allele specific primer extention, genetic bit analysis oligonucleotide-ligation assay, allele specific ligation chain reaction and sequencing techniques.

36. Method according to claim 35 characterized in that said evaluation is performed by techniques based on the use of restriction enzymes , allele specific PCR, hybridization techniques, or sequencing techniques.

37. Method according to claim 36 where said restriction enzymes are choosen among the following: TspR1, BsmI, PvuII.

38. Method for the in vitro diagnosis of Hereditary Hemochromatosis in a mammal comprising the evaluation of the presence in a biological sample obtained by said mammal of a mutated ferroportin 1 protein according to claim 21, where the presence of said protein is an indication that said mammal is affected by Hereditary Hemochromatosis.

39. Method according to claim 38 where said identification is performed by using antibodies able to specifically detect said mutated ferroportin 1 protein.

40. Monoclonal or policvlonal antibodies able to specifically detect a mutated ferroportin1 protein according to claims 21-22.

41. Use of antibodies according to claim 40 for the specific inactivation of a mutated ferroportin 1 protein according to the claim 21.

42. “computer readable” support characterized by comprising al least polinucleotides according to claims 1-17.

43. Use of polinucleotides according to claims 1-17 for the detection of polymorphisms in the ferroportin gene.

44. Use of polynucleotides according to claims 1-17 for the preparation of a pharmaceutical composition for the treatment of impaired iron homeostasis disease.

45. Use of polynucleotides according to claims 1-17 to modulate the expression of the gene coding for a mutated ferroportin1.

46. Diagnostic kit for non-HFE hereditary Hemochromatosis comprising at least one of the oligonucleotides according to claims 1-17.

47. Diagnostic kit for hereditary impaired ion homeostasis comprising at least one of the polynucleotides according to claims 1-17.

48. Diagnostic kit for polymorphisms of at least one of the chosen polymorphisms among the following: polymorphism of the nucleotide corresponding to position 238, polymorphism of the nucleotide corresponding to

position 521, polymorphism of the nucleotide corresponding to position 744 of sequence IDN1 characterized by comprising at least one of the oliglonucleotides of sequence: IDN13, IDN 14, IDN19, IDN20 combined with at least one of the following restriction enzymes:TspR1, Bsml,PvuII.

(SM/pd)

Milano, 09 June 2003

On behalf of PIETRANGELO Antonello

The Representative

Dr. Gemma Grevasi

NOTARBARTOLO & GERVASI S.p.A.

SEQUENCE LISTING

<110> Pietrangelo, Antonello

<120> Mutations in the SLC40A1 gene associated to impaired iron homeostasis

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Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
20 25 30

tct ctc tct act tgg gga gat cgg atg tgg cac ttt gcg gtg tct gtg 144
Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
35 40 45

ttt ctg gta gag ctc tat gga aac agc ctc ctt ttg aca gca gtc tac 192
Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
50 55 60

ggg ctg gtg gtg gca ggg tct gtt ctg gtc ctg gga gcc atc atc ggt 240
Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65 70 75 80

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Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
85 90 95

gtg gta cag aat gtt tca gtc atc ctg tgt gga atc atc ctg atg atg 336
Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
100 105 110

gtt ttc tta cat aaa cat gag ctt ctg acc atg tac cat gga tgg gtt 384
Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

ctc act tcc tgc tat atc ctg atc atc act att gca aat att gca aat 432
Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

ttg gcc agt act gct act gca atc aca atc caa agg gat tgg att gtt 480
Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145 150 155 160

gtt gtt gca gga gaa gac aga agc aaa cta gca aat atg aat gcc aca 528

Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
165 170 175

ata cga agg att gac cag tta acc aac atc tta gcc ccc atg gct gtt 576
Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
180 185 190

ggc cag att atg aca ttt ggc tcc cca gtc atc ggc tgt ggc ttt att 624
Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
195 200 205

tcg gga tgg aac ttg gta tcc atg tgc gtg gag tac gtc ctg ctc tgg 672
Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
210 215 220

aag gtt tac cag aaa acc cca gct cta gct gtg aaa gct ggt ctt aaa 720
Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225 230 235 240

gaa gag gaa act gaa ttg aaa cag ctg aat tta cac aaa gat act gag 768
Glu Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
245 250 255

cca aaa ccc ctg gag gga act cat cta atg ggt gtg aaa gac tct aac 816
Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
260 265 270

atc cat gag ctt gaa cat gag caa gag cct act tgt gcc tcc cag atg 864
Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

gct gag ccc ttc cgt acc ttc cga gat gga tgg gtc tcc tac tac aac 912
Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

cag cct gtg ttt ctg gct ggc atg ggt ctt gct ttc ctt tat atg act 960
Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

gtc ctg ggc ttt gac tgc atc acc aca ggg tac gcc tac act cag gga 1008
Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

ctg agt ggt tcc atc ctc agt att ttg atg gga gca tca gct ata act 1056
Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

gga ata atg gga act gta gct ttt act tgg cta cgt cga aaa tgt ggt 1104
Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
355 360 365

ttg gtt cgg aca ggt ctg atc tca gga ttg gca cag ctt tcc tgt ttg 1152
Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
370 375 380

atc ttg tgt gtg atc tct gta ttc atg cct gga agc ccc ctg gac ttg 1200
Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385 390 395 400

tcc gtt tct cct ttt gaa gat atc cga tca agg ttc att caa gga gag 1248
Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
405 410 415

tca att aca cct acc aag ata cct gaa att aca act gaa ata tac atg 1296
Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

tct aat ggg tct aat tct gct aat att gtc ccg gag aca agt cct gaa 1344

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Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
        435                 440                 445

tct gtg ccc ata atc tct gtc agt ctg ctg ttt gca ggc gtc att gct      1392
Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
    450                 455                 460

gct aga atc ggt ctt tgg tcc ttt gat tta act gtg aca cag ttg ctg      1440
Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465                 470                 475                 480

caa gaa aat gta att gaa tct gaa aga ggc att ata aat ggt gta cag      1488
Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
                485                 490                 495

aac tcc atg aac tat ctt ctt gat ctt ctg cat ttc atc atg gtc atc      1536
Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
            500                 505                 510

ctg gct cca aat cct gaa gct ttt ggc ttg ctc gta ttg att tca gtc      1584
Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
        515                 520                 525

tcc ttt gtg gca atg ggc cac att atg tat ttc cga ttt gcc caa aat      1632
Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
    530                 535                 540

act ctg gga aac aag ctc ttt gct tgc ggt cct gat gca aaa gaa gtt      1680
Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545                 550                 555                 560

agg aag gaa aat caa gca aat aca tct gtt gtt tga                      1716
Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
                565                 570


<210>  2
<211>  571
<212>  PRT
<213>  Homo sapiens

<400>  2

Met Thr Arg Ala Gly Asp His Asn Arg Gln Arg Gly Cys Cys Gly Ser
1               5                   10                  15


Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
            20                  25                  30


Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
        35                  40                  45


Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
    50                  55                  60


Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65                  70                  75                  80


Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
                85                  90                  95


Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
```

100 105 110

Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145 150 155 160

Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
165 170 175

Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
180 185 190

Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
195 200 205

Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
210 215 220

Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225 230 235 240

Glu Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
245 250 255

Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
260 265 270

Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
355 360 365

Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu

370   375   380

Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385   390   395   400

Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
                405   410   415

Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
            420   425   430

Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
        435   440   445

Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
    450   455   460

Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465   470   475   480

Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
                485   490   495

Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
            500   505   510

Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
        515   520   525

Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
    530   535   540

Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545   550   555   560

Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
                565   570

<210>   3
<211>   1716
<212>   DNA
<213>   Homo sapiens

<220>
<221>   CDS
<222>   (1)..(1716)
<223>   cDNA encoding a ferroportin 1 mutated in position (G80).

<400>   3
atg acc agg gcg gga gat cac aac cgc cag aga gga tgc tgt gga tcc   48
Met Thr Arg Ala Gly Asp His Asn Arg Gln Arg Gly Cys Cys Gly Ser
1   5   10   15

ttg gcc gac tac ctg acc tct gca aaa ttc ctt ctc tac ctt ggt cat   96

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Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
            20                  25                  30

tct ctc tct act tgg gga gat cgg atg tgg cac ttt gcg gtg tct gtg      144
Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
        35                  40                  45

ttt ctg gta gag ctc tat gga aac agc ctc ctt ttg aca gca gtc tac      192
Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
    50                  55                  60

ggg ctg gtg gtg gca ggg tct gtt ctg gtc ctg gga gcc atc atc agt      240
Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Ser
65                  70                  75                  80

gac tgg gtg gac aag aat gct aga ctt aaa gtg gcc cag acc tcg ctg      288
Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
                85                  90                  95

gtg gta cag aat gtt tca gtc atc ctg tgt gga atc atc ctg atg atg      336
Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
            100                 105                 110

gtt ttc tta cat aaa cat gag ctt ctg acc atg tac cat gga tgg gtt      384
Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
        115                 120                 125

ctc act tcc tgc tat atc ctg atc atc act att gca aat att gca aat      432
Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
    130                 135                 140

ttg gcc agt act gct act gca atc aca atc caa agg gat tgg att gtt      480
Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145                 150                 155                 160

gtt gtt gca gga gaa gac aga agc aaa cta gca aat atg aat gcc aca      528
Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
                165                 170                 175

ata cga agg att gac cag tta acc aac atc tta gcc ccc atg gct gtt      576
Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
            180                 185                 190

ggc cag att atg aca ttt ggc tcc cca gtc atc ggc tgt ggc ttt att      624
Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
        195                 200                 205

tcg gga tgg aac ttg gta tcc atg tgc gtg gag tac gtc ctg ctc tgg      672
Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
    210                 215                 220

aag gtt tac cag aaa acc cca gct cta gct gtg aaa gct ggt ctt aaa      720
Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225                 230                 235                 240

gaa gag gaa act gaa ttg aaa cag ctg aat tta cac aaa gat act gag      768
Glu Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
                245                 250                 255

cca aaa ccc ctg gag gga act cat cta atg ggt gtg aaa gac tct aac      816
Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
            260                 265                 270

atc cat gag ctt gaa cat gag caa gag cct act tgt gcc tcc cag atg      864
Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
        275                 280                 285

gct gag ccc ttc cgt acc ttc cga gat gga tgg gtc tcc tac tac aac      912
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Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
    290                 295                 300

cag cct gtg ttt ctg gct ggc atg ggt ctt gct ttc ctt tat atg act       960
Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305                 310                 315                 320

gtc ctg ggc ttt gac tgc atc acc aca ggg tac gcc tac act cag gga      1008
Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
                325                 330                 335

ctg agt ggt tcc atc ctc agt att ttg atg gga gca tca gct ata act      1056
Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
            340                 345                 350

gga ata atg gga act gta gct ttt act tgg cta cgt cga aaa tgt ggt      1104
Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
        355                 360                 365

ttg gtt cgg aca ggt ctg atc tca gga ttg gca cag ctt tcc tgt ttg      1152
Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
    370                 375                 380

atc ttg tgt gtg atc tct gta ttc atg cct gga agc ccc ctg gac ttg      1200
Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385                 390                 395                 400

tcc gtt tct cct ttt gaa gat atc cga tca agg ttc att caa gga gag      1248
Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
                405                 410                 415

tca att aca cct acc aag ata cct gaa att aca act gaa ata tac atg      1296
Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
            420                 425                 430

tct aat ggg tct aat tct gct aat att gtc ccg gag aca agt cct gaa      1344
Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
        435                 440                 445

tct gtg ccc ata atc tct gtc agt ctg ctg ttt gca ggc gtc att gct      1392
Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
    450                 455                 460

gct aga atc ggt ctt tgg tcc ttt gat tta act gtg aca cag ttg ctg      1440
Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465                 470                 475                 480

caa gaa aat gta att gaa tct gaa aga ggc att ata aat ggt gta cag      1488
Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
                485                 490                 495

aac tcc atg aac tat ctt ctt gat ctt ctg cat ttc atc atg gtc atc      1536
Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
            500                 505                 510

ctg gct cca aat cct gaa gct ttt ggc ttg ctc gta ttg att tca gtc      1584
Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
        515                 520                 525

tcc ttt gtg gca atg ggc cac att atg tat ttc cga ttt gcc caa aat      1632
Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
    530                 535                 540

act ctg gga aac aag ctc ttt gct tgc ggt cct gat gca aaa gaa gtt      1680
Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545                 550                 555                 560

agg aag gaa aat caa gca aat aca tct gtt gtt tga                      1716
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Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
565 570

<210> 4
<211> 571
<212> PRT
<213> Homo sapiens

<400> 4

Met Thr Arg Ala Gly Asp His Asn Arg Gln Arg Gly Cys Cys Gly Ser
1 5 10 15

Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
20 25 30

Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
35 40 45

Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
50 55 60

Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Ser
65 70 75 80

Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
85 90 95

Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
100 105 110

Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145 150 155 160

Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
165 170 175

Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
180 185 190

Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
195 200 205

Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
210 215 220

Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys

230 235 240

[illegible] Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
245 250 255

Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
260 265 270

Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
355 360 365

Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
370 375 380

Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385 390 395 400

Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
405 410 415

Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
435 440 445

Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
450 455 460

Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465 470 475 480

Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
485 490 495

Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile

500 505 510

Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
515 520 525

Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
530 535 540

Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545 550 555 560

Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
565 570

<210> 5
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<212> DNA
<213> Homo sapiens

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<222> (1)..(1716)
<223> cDNAencoding a ferroportin 1 mutated in position 174 (N174)

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Met Thr Arg Ala Gly Asp His Asn Arg Gln Arg Gly Cys Cys Gly Ser
1 5 10 15

ttg gcc gac tac ctg acc tct gca aaa ttc ctt ctc tac ctt ggt cat 96
Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
20 25 30

tct ctc tct act tgg gga gat cgg atg tgg cac ttt gcg gtg tct gtg 144
Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
35 40 45

ttt ctg gta gag ctc tat gga aac agc ctc ctt ttg aca gca gtc tac 192
Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
50 55 60

ggg ctg gtg gtg gca ggg tct gtt ctg gtc ctg gga gcc atc atc ggt 240
Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65 70 75 80

gac tgg gtg gac aag aat gct aga ctt aaa gtg gcc cag acc tcg ctg 288
Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
85 90 95

gtg gta cag aat gtt tca gtc atc ctg tgt gga atc atc ctg atg atg 336
Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
100 105 110

gtt ttc tta cat aaa cat gag ctt ctg acc atg tac cat gga tgg gtt 384
Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

ctc act tcc tgc tat atc ctg atc atc act att gca aat att gca aat 432
Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

ttg gcc agt act gct act gca atc aca atc caa agg gat tgg att gtt 480

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Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145                 150                 155                 160

gtt gtt gca gga gaa gac aga agc aaa cta gca aat atg att gcc aca      528
Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Ile Ala Thr
            165                 170                 175

ata cga agg att gac cag tta acc aac atc tta gcc ccc atg gct gtt      576
Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
        180                 185                 190

ggc cag att atg aca ttt ggc tcc cca gtc atc ggc tgt ggc ttt att      624
Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
    195                 200                 205

tcg gga tgg aac ttg gta tcc atg tgc gtg gag tac gtc ctg ctc tgg      672
Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
    210                 215                 220

aag gtt tac cag aaa acc cca gct cta gct gtg aaa gct ggt ctt aaa      720
Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225                 230                 235                 240

gaa gag gaa act gaa ttg aaa cag ctg aat tta cac aaa gat act gag      768
Glu Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
                245                 250                 255

cca aaa ccc ctg gag gga act cat cta atg ggt gtg aaa gac tct aac      816
Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
            260                 265                 270

atc cat gag ctt gaa cat gag caa gag cct act tgt gcc tcc cag atg      864
Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
        275                 280                 285

gct gag ccc ttc cgt acc ttc cga gat gga tgg gtc tcc tac tac aac      912
Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
    290                 295                 300

cag cct gtg ttt ctg gct ggc atg ggt ctt gct ttc ctt tat atg act      960
Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305                 310                 315                 320

gtc ctg ggc ttt gac tgc atc acc aca ggg tac gcc tac act cag gga     1008
Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
                325                 330                 335

ctg agt ggt tcc atc ctc agt att ttg atg gga gca tca gct ata act     1056
Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
            340                 345                 350

gga ata atg gga act gta gct ttt act tgg cta cgt cga aaa tgt ggt     1104
Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
        355                 360                 365

ttg gtt cgg aca ggt ctg atc tca gga ttg gca cag ctt tcc tgt ttg     1152
Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
    370                 375                 380

atc ttg tgt gtg atc tct gta ttc atg cct gga agc ccc ctg gac ttg     1200
Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385                 390                 395                 400

tcc gtt tct cct ttt gaa gat atc cga tca agg ttc att caa gga gag     1248
Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
                405                 410                 415

tca att aca cct acc aag ata cct gaa att aca act gaa ata tac atg     1296
```

Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

tct aat ggg tct aat tct gct aat att gtc ccg gag aca agt cct gaa 1344
Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
435 440 445

tct gtg ccc ata atc tct gtc agt ctg ctg ttt gca ggc gtc att gct 1392
Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
450 455 460

gct aga atc ggt ctt tgg tcc ttt gat tta act gtg aca cag ttg ctg 1440
Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465 470 475 480

caa gaa aat gta att gaa tct gaa aga ggc att ata aat ggt gta cag 1488
Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
485 490 495

aac tcc atg aac tat ctt ctt gat ctt ctg cat ttc atc atg gtc atc 1536
Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
500 505 510

ctg gct cca aat cct gaa gct ttt ggc ttg ctc gta ttg att tca gtc 1584
Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
515 520 525

tcc ttt gtg gca atg ggc cac att atg tat ttc cga ttt gcc caa aat 1632
Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
530 535 540

act ctg gga aac aag ctc ttt gct tgc ggt cct gat gca aaa gaa gtt 1680
Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545 550 555 560

agg aag gaa aat caa gca aat aca tct gtt gtt tga 1716
Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
565 570

<210> 6
<211> 571
<212> PRT
<213> Homo sapiens

<400> 6

Met Thr Arg Ala Gly Asp His Asn Arg Gln Arg Gly Cys Cys Gly Ser
1 5 10 15

Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
20 25 30

Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
35 40 45

Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
50 55 60

Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65 70 75 80

Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu

85 90 95

Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
100 105 110

Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145 150 155 160

Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Ile Ala Thr
165 170 175

Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
180 185 190

Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
195 200 205

Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
210 215 220

Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225 230 235 240

Glu Glu Glu Thr Glu Leu Lys Gln Leu Asn Leu His Lys Asp Thr Glu
245 250 255

Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
260 265 270

Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly

355 360 365

Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
370 375 380

Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385 390 395 400

Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
405 410 415

Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
435 440 445

Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
450 455 460

Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465 470 475 480

Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
485 490 495

Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
500 505 510

Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
515 520 525

Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
530 535 540

Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545 550 555 560

Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
565 570

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<211> 1716
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
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<223> cDNA encoding a ferroportina 1 mutated in position 248 (Q248).

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ttg gcc gac tac ctg acc tct gca aaa ttc ctt ctc tac ctt ggt cat      96
Leu Ala Asp Tyr Leu Thr Ser Ala Lys Phe Leu Leu Tyr Leu Gly His
            20                  25                  30

tct ctc tct act tgg gga gat cgg atg tgg cac ttt gcg gtg tct gtg      144
Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
        35                  40                  45

ttt ctg gta gag ctc tat gga aac agc ctc ctt ttg aca gca gtc tac      192
Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
    50                  55                  60

ggg ctg gtg gtg gca ggg tct gtt ctg gtc ctg gga gcc atc atc ggt      240
Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65                  70                  75                  80

gac tgg gtg gac aag aat gct aga ctt aaa gtg gcc cag acc tcg ctg      288
Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
                85                  90                  95

gtg gta cag aat gtt tca gtc atc ctg tgt gga atc atc ctg atg atg      336
Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
            100                 105                 110

gtt ttc tta cat aaa cat gag ctt ctg acc atg tac cat gga tgg gtt      384
Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
        115                 120                 125

ctc act tcc tgc tat atc ctg atc atc act att gca aat att gca aat      432
Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
    130                 135                 140

ttg gcc agt act gct act gca atc aca atc caa agg gat tgg att gtt      480
Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145                 150                 155                 160

gtt gtt gca gga gaa gac aga agc aaa cta gca aat atg aat gcc aca      528
Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
                165                 170                 175

ata cga agg att gac cag tta acc aac atc tta gcc ccc atg gct gtt      576
Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
            180                 185                 190

ggc cag att atg aca ttt ggc tcc cca gtc atc ggc tgt ggc ttt att      624
Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
        195                 200                 205

tcg gga tgg aac ttg gta tcc atg tgc gtg gag tac gtc ctg ctc tgg      672
Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp
    210                 215                 220

aag gtt tac cag aaa acc cca gct cta gct gtg aaa gct ggt ctt aaa      720
Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225                 230                 235                 240

gaa gag gaa act gaa ttg aaa cat ctg aat tta cac aaa gat act gag      768
Glu Glu Glu Thr Glu Leu Lys His Leu Asn Leu His Lys Asp Thr Glu
                245                 250                 255

cca aaa ccc ctg gag gga act cat cta atg ggt gtg aaa gac tct aac      816
Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
            260                 265                 270

atc cat gag ctt gaa cat gag caa gag cct act tgt gcc tcc cag atg      864
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Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

gct gag ccc ttc cgt acc ttc cga gat gga tgg gtc tcc tac tac aac 912
Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

cag cct gtg ttt ctg gct ggc atg ggt ctt gct ttc ctt tat atg act 960
Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

gtc ctg ggc ttt gac tgc atc acc aca ggg tac gcc tac act cag gga 1008
Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

ctg agt ggt tcc atc ctc agt att ttg atg gga gca tca gct ata act 1056
Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

gga ata atg gga act gta gct ttt act tgg cta cgt cga aaa tgt ggt 1104
Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
355 360 365

ttg gtt cgg aca ggt ctg atc tca gga ttg gca cag ctt tcc tgt ttg 1152
Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
370 375 380

atc ttg tgt gtg atc tct gta ttc atg cct gga agc ccc ctg gac ttg 1200
Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385 390 395 400

tcc gtt tct cct ttt gaa gat atc cga tca agg ttc att caa gga gag 1248
Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
405 410 415

tca att aca cct acc aag ata cct gaa att aca act gaa ata tac atg 1296
Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

tct aat ggg tct aat tct gct aat att gtc ccg gag aca agt cct gaa 1344
Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
435 440 445

tct gtg ccc ata atc tct gtc agt ctg ctg ttt gca ggc gtc att gct 1392
Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
450 455 460

gct aga atc ggt ctt tgg tcc ttt gat tta act gtg aca cag ttg ctg 1440
Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465 470 475 480

caa gaa aat gta att gaa tct gaa aga ggc att ata aat ggt gta cag 1488
Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln
485 490 495

aac tcc atg aac tat ctt ctt gat ctt ctg cat ttc atc atg gtc atc 1536
Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
500 505 510

ctg gct cca aat cct gaa gct ttt ggc ttg ctc gta ttg att tca gtc 1584
Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
515 520 525

tcc ttt gtg gca atg ggc cac att atg tat ttc cga ttt gcc caa aat 1632
Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
530 535 540

act ctg gga aac aag ctc ttt gct tgc ggt cct gat gca aaa gaa gtt 1680

Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
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20 25 30

Ser Leu Ser Thr Trp Gly Asp Arg Met Trp His Phe Ala Val Ser Val
35 40 45

Phe Leu Val Glu Leu Tyr Gly Asn Ser Leu Leu Leu Thr Ala Val Tyr
50 55 60

Gly Leu Val Val Ala Gly Ser Val Leu Val Leu Gly Ala Ile Ile Gly
65 70 75 80

Asp Trp Val Asp Lys Asn Ala Arg Leu Lys Val Ala Gln Thr Ser Leu
85 90 95

Val Val Gln Asn Val Ser Val Ile Leu Cys Gly Ile Ile Leu Met Met
100 105 110

Val Phe Leu His Lys His Glu Leu Leu Thr Met Tyr His Gly Trp Val
115 120 125

Leu Thr Ser Cys Tyr Ile Leu Ile Ile Thr Ile Ala Asn Ile Ala Asn
130 135 140

Leu Ala Ser Thr Ala Thr Ala Ile Thr Ile Gln Arg Asp Trp Ile Val
145 150 155 160

Val Val Ala Gly Glu Asp Arg Ser Lys Leu Ala Asn Met Asn Ala Thr
165 170 175

Ile Arg Arg Ile Asp Gln Leu Thr Asn Ile Leu Ala Pro Met Ala Val
180 185 190

Gly Gln Ile Met Thr Phe Gly Ser Pro Val Ile Gly Cys Gly Phe Ile
195 200 205

Ser Gly Trp Asn Leu Val Ser Met Cys Val Glu Tyr Val Leu Leu Trp

210 215 220

Lys Val Tyr Gln Lys Thr Pro Ala Leu Ala Val Lys Ala Gly Leu Lys
225 230 235 240

Glu Glu Glu Thr Glu Leu Lys His Leu Asn Leu His Lys Asp Thr Glu
245 250 255

Pro Lys Pro Leu Glu Gly Thr His Leu Met Gly Val Lys Asp Ser Asn
260 265 270

Ile His Glu Leu Glu His Glu Gln Glu Pro Thr Cys Ala Ser Gln Met
275 280 285

Ala Glu Pro Phe Arg Thr Phe Arg Asp Gly Trp Val Ser Tyr Tyr Asn
290 295 300

Gln Pro Val Phe Leu Ala Gly Met Gly Leu Ala Phe Leu Tyr Met Thr
305 310 315 320

Val Leu Gly Phe Asp Cys Ile Thr Thr Gly Tyr Ala Tyr Thr Gln Gly
325 330 335

Leu Ser Gly Ser Ile Leu Ser Ile Leu Met Gly Ala Ser Ala Ile Thr
340 345 350

Gly Ile Met Gly Thr Val Ala Phe Thr Trp Leu Arg Arg Lys Cys Gly
355 360 365

Leu Val Arg Thr Gly Leu Ile Ser Gly Leu Ala Gln Leu Ser Cys Leu
370 375 380

Ile Leu Cys Val Ile Ser Val Phe Met Pro Gly Ser Pro Leu Asp Leu
385 390 395 400

Ser Val Ser Pro Phe Glu Asp Ile Arg Ser Arg Phe Ile Gln Gly Glu
405 410 415

Ser Ile Thr Pro Thr Lys Ile Pro Glu Ile Thr Thr Glu Ile Tyr Met
420 425 430

Ser Asn Gly Ser Asn Ser Ala Asn Ile Val Pro Glu Thr Ser Pro Glu
435 440 445

Ser Val Pro Ile Ile Ser Val Ser Leu Leu Phe Ala Gly Val Ile Ala
450 455 460

Ala Arg Ile Gly Leu Trp Ser Phe Asp Leu Thr Val Thr Gln Leu Leu
465 470 475 480

Gln Glu Asn Val Ile Glu Ser Glu Arg Gly Ile Ile Asn Gly Val Gln

485 490 495

Asn Ser Met Asn Tyr Leu Leu Asp Leu Leu His Phe Ile Met Val Ile
500 505 510

Leu Ala Pro Asn Pro Glu Ala Phe Gly Leu Leu Val Leu Ile Ser Val
515 520 525

Ser Phe Val Ala Met Gly His Ile Met Tyr Phe Arg Phe Ala Gln Asn
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Thr Leu Gly Asn Lys Leu Phe Ala Cys Gly Pro Asp Ala Lys Glu Val
545 550 555 560

Arg Lys Glu Asn Gln Ala Asn Thr Ser Val Val
565 570

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<213> Homo sapiens

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cagctcatta agtgactacc atcgc 25

<210> 12
<211> 24

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<400> 12
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<211> 23
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<210> 14
<211> 22
<212> DNA
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<223> 3' PCR primer. Exon 3

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<210> 19
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<220>
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<223> 3' PCR primer.Exon 6

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ccccactggt aataaaacct g 21

<210> 21
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<400> 21
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<210> 22
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<210> 23
<211> 24
<212> DNA
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<400> 23

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<210> 24
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<212> DNA
<213> Homo sapiens

<400> 24
gctgacttag gtttcctaaa cagc 24

<210> 25
<211> 10
<212> DNA
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<220>
<221> misc_feature
<223> oligonucleotide comprising the polymorphism at nt 238

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<210> 26
<211> 10
<212> DNA
<213> Homo sapiens

<220>
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<223> oligonucleotide comprising the polymorphism at nt 521.

<400> 26
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<210> 27
<211> 10
<212> DNA
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<220>
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<223> oligonucleotide comprising the polymorphism at nt 744

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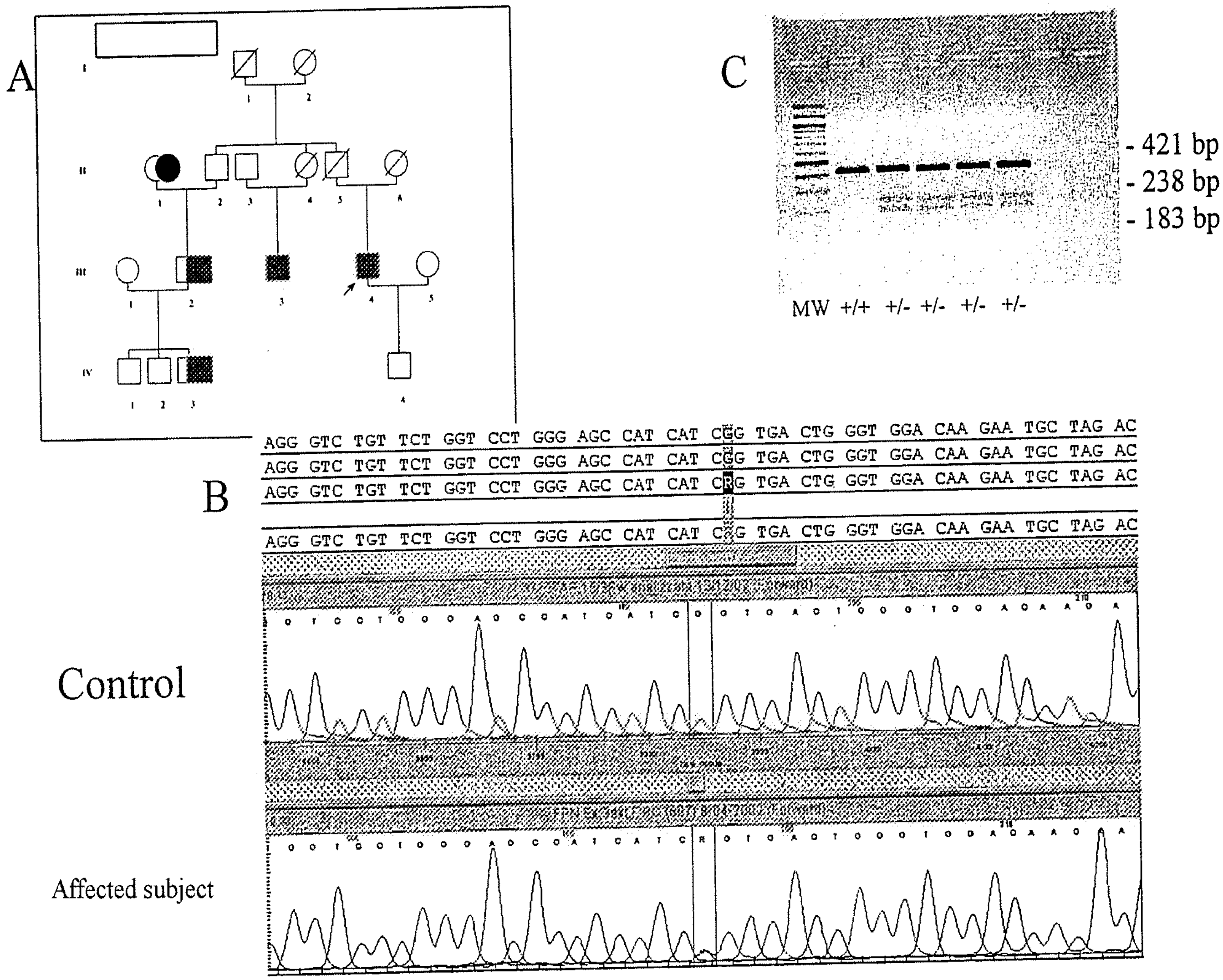
gaaacatctg 10

Figure 1


A
I
II
III
IV
B
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AGG GTC TGT TCT GGT CCT GGG AGC CAT CAT CRG TGA CTG GGT GGA CAA GAA TGC TAG AC
AGG GTC TGT TCT GGT CCT GGG AGC CAT CAT CGG TGA CTG GGT GGA CAA GAA TGC TAG AC
Control
Affected subject
C
- 421 bp
- 238 bp
- 183 bp
MW +/+ +/- +/- +/- +/-

Figure 2

A

Control

Affected subject

B

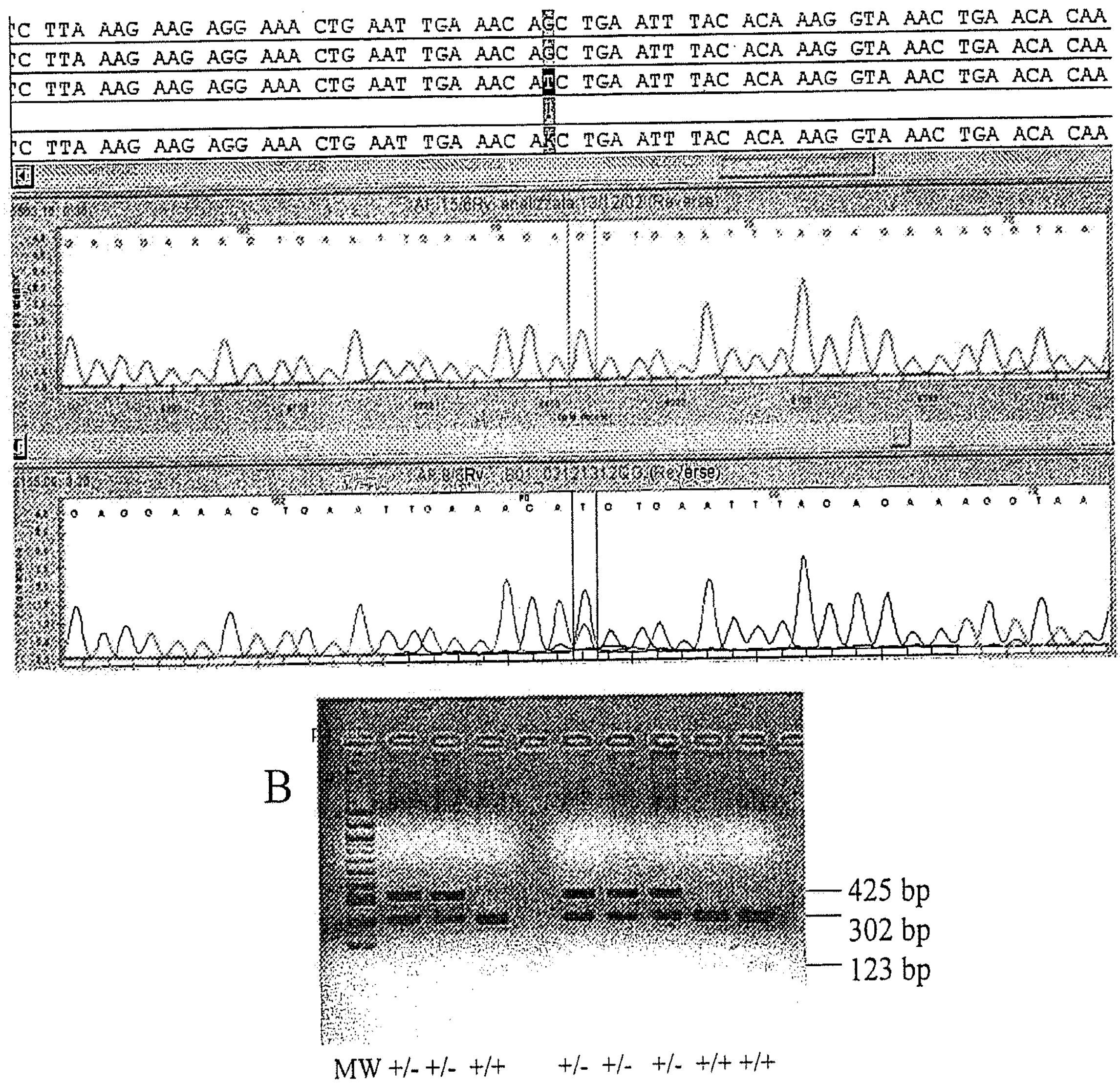
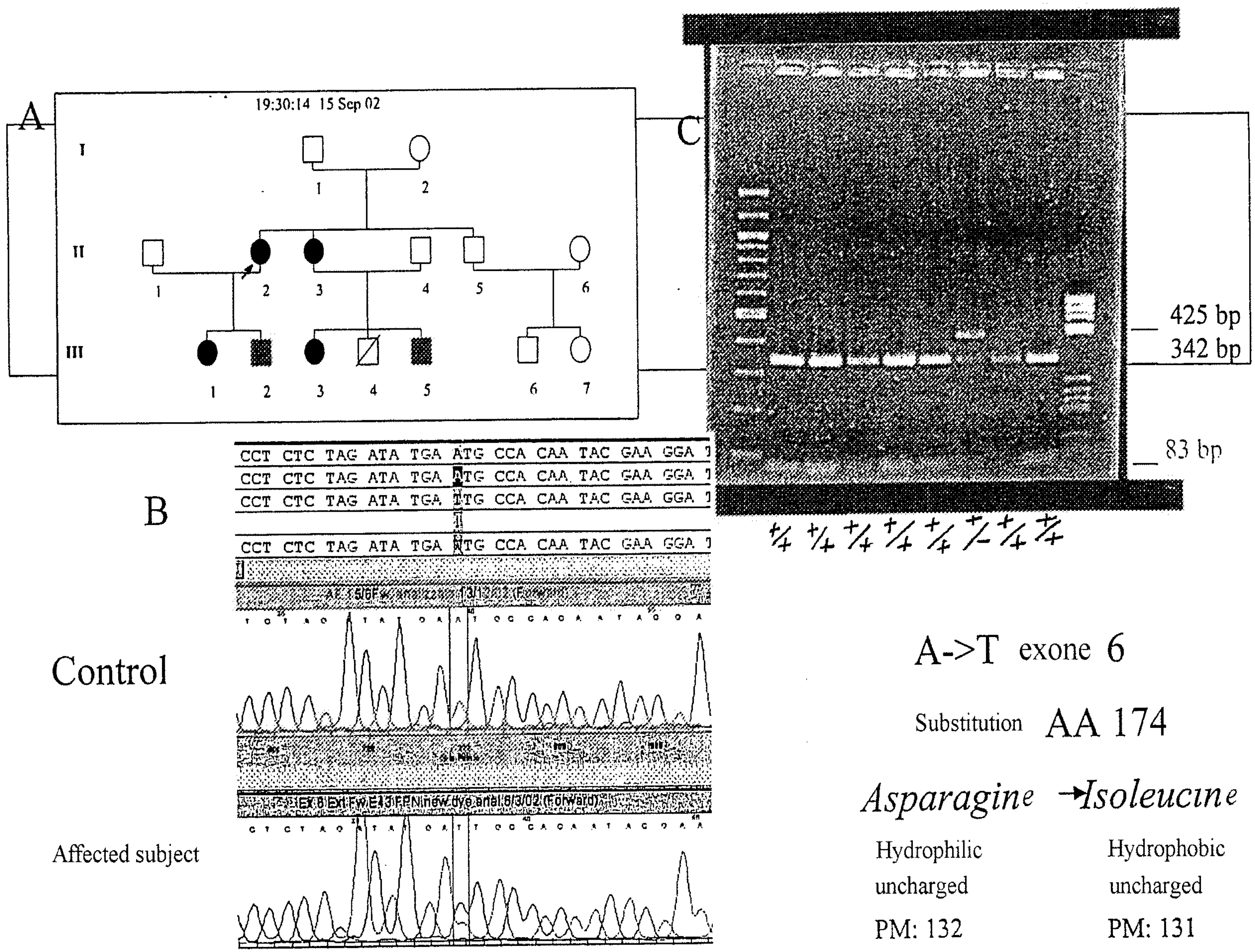


Figure 3


A
19:30:14 15 Sep 02
I
II
III
B
CCT CTC TAG ATA TGA ATG CCA CAA TAC GAA GGA
Control
Affected subject
C
425 bp
342 bp
83 bp
+/+ +/+ +/+ +/+ +/+ +/- +/+ +/+
A->T exone 6
Substitution AA 174
Asparagine →Isoleucine
Hydrophilic
uncharged
PM: 132
Hydrophobic
uncharged
PM: 131